

ART. X.—*A Method of Estimating Small Amounts of Calcium.*

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(Communicated by Professor W. A. Osborne).

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For long there has been need of a simple and easy method of estimating small quantities of calcium, such as would be useful in clinical medicine. After some 'four years' experimenting the following method was found to be the most useful:—

To a slightly acid solution containing a soluble calcium salt, from three-quarters to an equal volume of methylated spirits is added without mixing, then a few drops of a saturated solution of oxalic acid, and the whole shaken up, almost immediately a white cloud appears.

The addition of small quantities of alcohol hastens the formation of calcium oxalate deposit, but on putting some of this white precipitate under the ultra-microscope no crystalline shape is observed, but small rounded masses about 0.1μ in diameter, and comparing them with a typhoid bacillus it was estimated it would take 128 to make up the same size.

In estimating the calcium content of the blood a small glass tube is marked to 1 c.c. content, and blood is drawn from a vein with a hypodermic syringe, and immediately transferred to the tube up to the 1 c.c. mark. This is placed at leisure in a platinum crucible, using a little distilled water to wash out the tube; the wash water is also transferred to the crucible. It is then passed to and fro through a strong flame, and with practice one is able to burn the blood to a black ash without any bubbling over or spluttering, which is inevitable if it is dried off first. It has also the advantage of being rapid. It is then placed in a small electric furnace, which is already at a dull red heat, and left there till thoroughly ashed, about half an hour being ample. During the ashing, controls and filter papers are prepared.

For the nephelometric method the writer uses glass tubes of an even bore with flat bottoms about 8 cm. long and marked in 1 c.c. gradations. Total capacity would be about 8 c.c. Double filter papers are used 4 cm. in diameter and very small glass funnels. The filter-papers are rinsed through with 4 c.c. of 2 per cent. acetic acid, which is found sufficient to eliminate all traces of soluble Ca from them. This is necessary, as Mr. H. Lyman

pointed out that all filter-papers, even the best Swedish, contain noticeable amounts of calcium. When the crucible is cool, 1 c.c. of 2 per cent. acetic acid is added by means of a graduated pipette with rubber teat. It is stirred round with a glass rod, rinsing the rod after each use. The contents are drawn up with a pipette and filtered, the same care being taken by rinsing the pipette each time. Another 1 c.c. of 2 per cent. acetic acid is again added to the crucible, and after agitating is filtered. 0.5 c.c. of distilled water is used to wash out the crucible, and then passed through the filter-papers, making a total of 2.5 c.c. in the tube. A solution of a calcium salt, phosphate or chloride in dilute acetic acid is used of the strength of 0.0025 grms. of calcium per 1 c.c., for controls 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2 c.c. are used, giving a wide range which represents 0.015, 0.0175, 0.02, 0.0225, 0.025, 0.0275, 0.03 mg. of calcium. These are placed in the tubes and filled up to 2.5 c.c. with distilled water.

Now all is ready for testing.

Add 1.5 c.c. of alcohol (ordinary methylated spirits, if calcium-free, will do), then three drops of a saturated solution of oxalic acid with a dropper. They are all shaken as rapidly as possible, and within a few minutes the results can be read by looking down the tubes and comparing with the controls.

A 10 per cent. variation even in such small quantities can be estimated by this somewhat crude nephelometric method, and with the advent of one of the new instruments finer grades will easily be obtained.

Since completing this method Mr. H. Lyman has shown that a large percentage of the calcium can be picked up by mixing the blood with twice or three times its volume of a 6.5 per cent. solution of trichloroacetic acid, and as a clinical test the following gives a fair degree of accuracy and is rapidly carried out.

Dilute a given quantity of blood with twice its volume of a 6.5 per cent. solution of trichloroacetic acid and allow to stand for twenty-four hours, by then there will be a clear fluid above the precipitated proteins, 2 c.c. of this is pipetted off and neutralised till faintly acid, it is transferred to the nephelometric tube and equal quantities of methylated spirit added, then three drops of a saturated solution of oxalic acid, and shaken. The result can be read against controls within a few minutes, giving one-third of 2 c.c. of blood.

My thanks are tendered to your President, Professor Osborne, for much advice and help during these investigations.